Lactic acid treatment in T cell culture

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An abbreviated version of this protocol was published in Science Translational Medicine in Oct 2020

Metabolic reprogramming of donor T cells enhances graft-versus-leukemia effects in mice and humans

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Detailed protocol

Pan T cell isolation and activation

- Isolate spleen
- Isolate T cells using the Pan T cell isolation kit from Miltenyi following the manufacturer's instructions
- Resuspend T cells in culture medium

Set up T cell culture

- Count T cells and adjust to $1x10^6$ T cells per 1 ml medium
- Wash CD3/CD28 Dynabeads as described in manufacturer's instructions and adjust to 25 µl beads for $1x10^6$ cells per 1 ml medium
- Mix Beads with T cells and immediately seed in desired format (tested: 96 well, 24 well, 12 well, 6 well)
- Directly add lactic acid at desired concentration (tested: 10 mM, 15 mM; stock 1 M in sterile H_2O)
- Incubate 48 h for metabolic measurements or cell cycle analysis, 72 h for proliferation analysis

Materials:

Culture medium	RPMI 1640 1x + L-Glutamine
	10% FCS
	100 U/ml Penicillin
	100 µg/ml Streptomycin
	25 µM β-mercaptoethanol

Pan T Cell Isolation Kit II, mouse	130-095-130	Miltenyi
Dynabeads Mouse T-Activator CD3/CD28	11453D	ThermoFisher
Lactic acid	L6402	Sigma

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Uhl, F. (2020). Lactic acid treatment in T cell culture. Bio-protocol Preprint. bio-protocol.org/prep631.

2. Uhl, F. M., Chen, S., O'Sullivan, D., Edwards-Hicks, J., Richter, G., Haring, E., Andrieux, G., Halbach, S., Apostolova, P., Büscher, J., Duquesne, S., Melchinger, W., Sauer, B., Shoumariyeh, K., Schmitt-Graeff, A., Kreutz, M., Lübbert, M., Duyster, J., Brummer, T., Boerries, M., Madl, T., Blazar, B. R., Groß, O., Pearce, E. L. and Zeiser, R.(2020). Metabolic reprogramming of donor T cells enhances graft-versus-leukemia effects in mice and humans. Science Translational Medicine 12(567). DOI: 10.1126/scitranslmed.abb8969

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